

The role of different soil sample digestion methods on trace elements analysis: a comparison of ICP-MS and INAA measurement results

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Abstract The measurement of trace-element concentration in soil, sediment and waste, is generally a combination of a digestion procedure for dissolution of elements and a subsequent measurement of the dissolved elements. “Partial” and “total” digestion methods can be used in environmental monitoring activities. To compare measurement results obtained by different methods, it is crucial to determine and to maintain control of the bias of the results obtained by these methods. In this paper, ICP-MS results obtained after matrix digestion with modified aqua regia ($\text{HCl} + \text{HNO}_3 + \text{H}_2\text{O}_2$) method and two “total” digestion methods (microwave aqua regia + HF and $\text{HNO}_3 + \text{HF}$) are compared with those obtained by instrumental neutron activation analysis, a non-destructive analytical method for the determination of the total mass concentrations of inorganic components in environmental matrices. The comparison was carried out on eight agricultural soil samples collected in one test area and measured by k_0 -INAA and ICP-MS to determine As, Co, Cr, Sb and Zn mass concentration. The bias of results for As, Cd, Co, Cr, Cu, Ni, Pb, Sb and Zn of the three digestion methods were assessed using selected measurement standards. This

paper highlights that the digestion procedure is an integral part of the measurement and can affect the measurement result in environmental analysis.

Keywords Digestion methods · Trace elements · Soils · INAA · ICP-MS

Introduction

Measurement of trace-element mass concentrations in soils is the first step in evaluating their potential health or ecological hazard. Sample digestion is often a necessary step before determining “total” element mass concentrations in soils. A standard and relatively safe dissolution method that provides an analytical recovery of at least approximately 90% of soil bound elements is required in most laboratories working on trace elements in soil.

Various digestion methods are used to determine the mass concentration of trace elements in solid matrices, including different combinations of concentrated acids [1–3]. Open beakers heated on hot plates, digestion tubes in a block digester, and digestion bombs placed in microwave ovens are the most commonly used equipment to digest solid sample matrices.

In particular, since the 1980s, the microwave-assisted sample digestion technique has become popular and presently it is widely used due to its safe, rapid and efficient performance [4–6].

Since different acid digestion methods applied to soil samples can release a different amount of elements from this matrix, it is critical to compare different digestion methods used to determine elemental mass fractions in soils. As reported by Chen and Lena [4], the amount of trace element extracted by the commonly used digestion

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methods might depend on the element, their origin (anthropogenic or natural), soil properties and element mass fractions.

The aqua regia digestion method (USEPA 3050 [7] or ISO standard 11466 [8]) is considered effective for measuring “total” trace element in soils and is usually used to give an estimate of the maximum element availability to plants [4, 9]. This method consists of treating a soil sample with a 3:1 mixture of hydrochloric (HCl) and nitric (HNO₃) acids. The nitric acid destroys organic matter and oxidizes sulphide material. It reacts with concentrated hydrochloric acid to generate aqua regia: $3\text{HCl} + \text{HNO}_3 \rightarrow 2 \text{H}_2\text{O} + \text{NOCl} + \text{Cl}_2$. Aqua regia is considered adequate for dissolving most base element sulphates, sulphides, oxides and carbonates but only provides a “partial” extraction for most rock forming elements and elements of a refractory nature. For example, aqua regia extraction might give complete recovery for Cd, Cu, Pb and Zn while it is known to provide partial recovery for Cr, Ni and Ba. The latter elements can only be efficiently recovered by using hydrofluoric acid (HF). However, aqua regia digestion method is internationally accepted to measure concentrations in soil, admitting that the fraction of elements not extracted by this method, as not available for biological uptake. The ISO standard on aqua regia digestion of soil includes only digestion by the use of hot plate heating, while the modified aqua regia digestion method, suggested by the Italian legislation, includes both hot-plate heating and microwave-oven heating [10]. The aqua regia modified method adds, in the first step, hydrogen peroxide (H₂O₂) in order to enhance the destruction of the organic matter in the soil.

More vigorous HNO₃ + HCl + HF digestion methods (like EN 13656 applied to wastes [11]) provide satisfactory dissolution of silica matrices [12–15]. These methods use microwave-assisted acid digestion for “total” sample decomposition and are applicable to up to 30 elements. HNO₃ + HCl + HF and HNO₃ + HF mixtures dissolve silica matrices due to the presence of HF, via the reaction: $\text{HF} + \text{SiO}_2 \rightarrow \text{H}_2\text{SiF}_6 + \text{H}_2\text{O}$.

In the present study, the three digestion methods reported above were compared for the analysis of eight agricultural soil samples collected at an Italian reference site, previously characterized within the framework of an APAT project focused on soil sampling uncertainty evaluation [16, 17]. The measurand elements (As, Cd, Co, Cr, Cu, Ni, Pb, Sb and Zn) were measured in the solutions of digested samples using inductively coupled plasma mass spectrometer (ICP-MS). Furthermore, five elements (As, Co, Cr, Sb, Zn) were measured by the *k*₀-standardization method of Instrumental Neutron Activation Analysis (*k*₀-INAA). INAA is a non-destructive analytical method for the measurement of inorganic components in solid matrices, because this method does not require any sample dissolution. INAA is a valu-

able technique particularly for elements that form or are in refractory phases that may be difficult to dissolve [18]. In this way, the effect of the dissolution step on the final measurement results for As, Co, Cr, Sb and Zn was investigated.

The difference between the reference values and the ICP-MS results on the solutions obtained in the laboratory with the three different digestion procedures was evaluated by the laboratory bias. The modified microwave aqua regia method was applied to two different certified reference materials (LGC-6187 and BCR-141R) characterized for hot aqua regia total-recoverable trace elements. The microwave aqua regia + HF and HNO₃ + HF methods were applied to digest two IAEA reference materials (SL-1 and Soil-7) characterized for total elements. IAEA Soil-7 was as well used as quality control material for INAA measurements.

Materials and methods

Sample collection

Eight soil samples were collected at an agricultural site, located in the North East of Italy (Pozzuolo del Friuli, Udine), within the framework of an APAT project [16]. Sampling was performed in June 2001, using strictly controlled protocols. The details of sampling are fully described in Barbizzi et al. [17]. The agricultural area sampled reveals a quite balanced soil grain size distribution with a slight dominance of the silt fraction (47%) and a low percentage of clay (below 16%). On average, the fraction above 2 mm represents only the 13% of the sampled soil. Relatively high pH values (about 7.7) and a low percentage of organic carbon content are observed. The cation exchange capacity (CEC) along the area, reveals low values (in average below 16 cmol₍₊₎ kg⁻¹). These are compatible both with the slight contribution derived by low clay content and the poor level of organic carbon.

Sample preparation

Soil samples were weighed and stored in cardboard boxes and then dried in an oven with a fan at 36–40°C until constant weight was reached. Then they were disaggregated using a wood pestle, sieved at 2 mm, the volume was reduced by quartering and riffing and at the end the laboratory samples were milled at 90 μm to obtain the test samples [19]. Barbizzi et al. [17] report in more detail the sample preparation steps. From each of the eight test samples, nine test portions [19] were taken for trace metals analysis by ICP-MS and 1 test portion was taken for INAA analysis. The homogeneity of the test samples has been tested by INAA, analyzing ten test portions from three different test samples.

Table 1 Microwave oven digestion cycles (methods A-B-C)

Method A HNO ₃ + HF		Method B HCl + HNO ₃ + HF		Method C HCl + HNO ₃ + H ₂ O ₂	
Time (min)	Power (W)	Time (min)	Power (W)	Time (min)	Power (W)
10	250	2	500	10	250
10	400	2	0	10	450
10	650	5	500	10	600
5	400	5	800	5	250
10	250	5	1,000		

Digestion methods

Microwave digestions were performed in a close microwave oven system (CEM Corporation Mars 5, Matthews, NC, USA).

For the three digestion methods, a test portion of about 0.1 g was weighed into a 120-mL Teflon-PFA microwave digestion vessel after manually shaking the bottles for at least 1 min. The HNO₃ + HF digestion (method A) was performed by adding to the soil a mixture of 3 mL of HNO₃ and 2 mL of HF.

The aqua regia + HF digestion (method B) used in the present work followed the EN 13656 method [11] developed for elemental measurement in wastes. A freshly prepared mixture of 2 mL HNO₃ + 6 mL HCl + 2 mL HF was added to the sample.

The modified aqua regia digestion followed the method suggested by the Italian legislation (method C) [10]. First 1.5 mL H₂O₂, 4.5 mL HCl and 1.5 mL HNO₃ were added to the soil. Hydrogen peroxide was used to enhance the destruction of organic matter.

All the samples were microwave digested following the digestion cycles reported in Table 1. The time integrated energy was 5,265 and 3,192 kJ, respectively, for methods A and B. Each digestion cycle was performed using the maximum number of vessels available (12 vessels).

The three digestion procedures were performed in triplicate for each test sample (CRMs, RMs and agricultural soil samples).

After digestion, each aliquot was quantitatively transferred to a volumetric flask (Brand) and diluted with MilliQ water to 100 mL. Before diluting, rhodium at a concentration of 10 µg/L was added as internal standard to minimize the instrumental signal fluctuation and matrix effects. The solutions were allowed to stand for 24 h without removing the undissolved residue and then analyzed by ICP-MS.

Nitric acid, hydrofluoric acid and hydrochloric acid of ultra-pure grade were purchased from Merck.

ICP-MS measurements

Trace metal mass fractions were measured on an Agilent technologies 7500c ICP-MS equipped with a collision cell to minimize polyatomic interferences and matrix effects.

Babington nebulizer, standard spray chamber, Cetac ASX 500 auto sampler was used in the measurements. The collision cell was pressurized with He gas (flow of 2.9 mL min⁻¹) to reduce interferences by dissociating interfering polyatomic species by collision and by charge transfer. The ICP-MS is optimized daily with a tuning solution at 10 µg L⁻¹ of Li, Ce, Y, Tl. Optimization is performed using normal mode and collision cell mode. A typical analytical run after optimization of the ICP-MS consists of calibration standard solutions, procedure blanks, samples and CRM. Calibration standard solutions are daily prepared from a working standard solution containing 5 µg mL⁻¹ of Cr and Zn, 0.5 µg mL⁻¹ of Co, 1.5 µg mL⁻¹ of Ni and Cu, 0.1 µg mL⁻¹ of Cd and Sb and 2.5 µg mL⁻¹ of Pb. The working standard solution was prepared from 1,000 µg mL⁻¹ stock solutions of all elements by dilution with ultra-pure water in a 100-mL volumetric flask. Calibration curve has been determined on five points for each element, in a range from 0 to 300 ng mL⁻¹ for Cr and Zn, 0 to 30 ng mL⁻¹ for Co, 0 to 90 ng mL⁻¹ for Ni and Cu, 0 to 6 ng mL⁻¹ for Cd and Sb and 0 to 150 ng mL⁻¹ for Pb. The calibration standard solutions are traceable to values embodied in measurement standards issued by the NIST.

The ⁷⁵As, ⁵⁹Co, ⁵²Cr, ⁶⁰Ni, ⁶³Cu, ⁶⁶Zn, ¹¹¹Cd, ¹²¹Sb and ²⁰⁸Pb isotopes were chosen for measurement. In the present work, He gas was demonstrated to effectively minimize the interferences of ⁴⁰Ar ³⁵Cl on the isotope ⁷⁵As, ⁴⁰Ar ¹²C on ⁵²Cr, ⁴⁰Ar ¹⁸O on the isotope ⁵⁹Co and ⁴⁴Ca ¹⁶O, ²³Na ³⁷Cl on the isotope ⁶⁰Ni. For the agricultural soils the collision cell was used only for ⁵⁹Co and ⁷⁵As. Two procedure blanks and two RMs were measured every eight samples.

k₀-INAA measurements

Measurement of As, Co, Cr, Sb and Zn by k₀-INAA were carried out at the Jožef Stefan Institute, Ljubljana, Slovenia. For details about k₀-INAA and the relevant nuclear data see Jačimović et al. [18].

Test portions of about 0.2 g (one for each test sample) were sealed into suprapure plastic containers and irradiated for about 20 h in the carousel facility of the TRIGA Mark II reactor, Ljubljana (thermal neutron flux 1.0 × 1,012 n cm⁻² s⁻¹). A 1.0-mm Al-0.1% Au alloy wire pressed into a disk (diameter of 6 mm, thickness 0.2 mm) was co-irradiated with the

Table 2 Element mass fractions measured by INAA in the RM IAEA Soil-7

IAEA Soil-7	Recommended value (95% confidence interval) (mg kg ⁻¹)	INAA (<i>n</i> = 14) (mg kg ⁻¹)
Cr	60 (49–74)	69.5 ± 5.2
Co	8.9 (8.4–10.1)	8.8 ± 0.6
Zn	104 (101–113)	103.1 ± 6.2
As	13.4 (12.5–14.2)	14.5 ± 0.6
Sb	1.7 (1.4–1.8)	1.8 ± 0.09

Note: Results are reported with their standard deviations at an approximate level of 95%. The table also reports the recommended values and the 95% confidence intervals for IAEA Soil-7 (*n* = number of independent replicates).

sample as a comparator. The irradiated samples were subsequently transferred to clean polyethylene vials and counted on calibrated coaxial HPGe detectors connected to a multi-channel analyzer (MCA). Each irradiated sample was measured three times: after 2–3, 8, and 30 days cooling time. *k*₀-INAA quality control was performed by using the measurement standard IAEA Soil-7. Results of quality control tests are reported in Table 2.

Laboratory bias determination

Measurement standards (RMs) such as IAEA SL-1 (lake sediment) and IAEA Soil-7 from the International Atomic Energy Agency (IAEA), Austria, and measurement standards (CRMs) BCR-141R (calcareous loam soil) from the European Commission, Belgium and LGC-6187 (river sediment) from the Laboratory of the Government Chemist (LGC), UK, were digested in triplicate following the procedures reported above. As previously stated, IAEA SL-1 and IAEA Soil-7 are characterized for total elements mass fractions and were processed using microwave aqua regia + HF and HNO₃ + HF. The measurement standards BCR-141R and LGC-6187 are supplied with certified values for extractable elements using methods based on DIN 38414-S7 and ISO11466, respectively, and were digested in this study by using the modified aqua regia procedure. The BCR-141R is certified for total mass fraction as well. Bias was judged by comparing the measured mass fraction with the certified/recommended values of the measurement standards [14].

Results presentation

In this paper, the terms repeatability, standard deviation, etc., refer to ISO 3534-1 [20]. Repeatability was defined as the ratio of the standard deviation to the average, obtained under repeatability conditions, expressed as relative standard deviation. These standard deviations, associated with the measurement values for the measurands, are multiplied by a cov-

erage factor of 2, to yield an approximate confidence level of 95%.

Analytical recovery is defined as the value observed divided by the value expected and multiplied by 100.

On the basis of the assumption that measurement results obtained on homogeneous material are distributed normally, the comparison between methods (A + B + C versus INAA and A + B versus C) was carried out using the grand mean, requiring data to be normally distributed. The assumption of normal distribution of the results is derived from inter-laboratory comparisons carried out by APAT, in which the normality of the distribution of the element mass fraction in sediment/compost RMs was verified on data from about 70 laboratories [21, 22].

Results and discussion

Laboratory bias and repeatability standard deviations in RMs and CRMs

In general, recoveries (% R) within 81 to 122% were obtained for all elements for the three digestion methods. Table 3 reports the results and associated standard deviations, obtained with methods A and B. A good agreement with the recommended values has been obtained for the Co, Ni, Cu, As, Sb and Cd mass fractions for both methods in the two RMs (IAEA Soil-7 and IAEA SL-1).

Mass fraction low recoveries for Cr even using the HF (method A and B) are described in the literature [23, 24] and are associated with the presence of insoluble refractory Cr minerals such as chromspinel and chromites (FeCr₂O₇). These minerals, frequently occurring in geological materials, are very difficult to dissolve and this behavior can result in low recovery. In this investigation, all values of “total” Cr mass fraction fall within the confidence intervals reported in the RM certificates. The lower efficiency of digestion methods in extracting Cr from soils can be detected aggregating by method the measurement results of the laboratories participating in the certification of IAEA Soil-7 and IAEA SL-1 [25, 26]. The mean values obtained for IAEA Soil-7 are 67 ± 6 mg kg⁻¹, for measurements by direct INAA method, and 50 ± 11 mg kg⁻¹, for measurements by atomic absorption spectrometry. These values are respectively higher and lower than the recommended value. The same behavior is shown for IAEA SL-1 where the aggregated values lead to 112 ± 19 mg kg⁻¹ for INAA measurements, and 89 ± 29 mg kg⁻¹ for measurements obtained by atomic absorption spectrometry.

The Zn mass fraction values measured with method A in IAEA SL-1 is significantly lower than the reference value. In the other cases, measured Zn mass fraction values fall within the 95% confidence interval of the RMs, but all values are lower than the reference value. Zn is generally brought into

Table 3 Element mass fractions for digestion methods A–B applied to IAEA Soil-7 and SL-1 RMs

	IAEA Soil-7 Recommended value (95% confidence interval) (mg kg ⁻¹)	IAEA SL-1 Recommended value (95% confidence interval) (mg kg ⁻¹)	Measured mass fractions			
			Method A HNO ₃ + HF		Method B HCl + HNO ₃ + HF	
			IAEA Soil-7 (n = 3) (mg kg ⁻¹)	IAEA SL-1 (n = 3) (mg kg ⁻¹)	IAEA Soil-7 (n = 3) (mg kg ⁻¹)	IAEA SL-1 (n = 3) (mg kg ⁻¹)
Cr	60 (49–74)	104* (95–113)	49 ± 10	109 ± 19	54 ± 16	106 ± 17
Co	8.9 (8.4–10.1)	19.8 (18.3–21.3)	9 ± 1	18 ± 3	9 ± 2	18 ± 4
Ni	26* (21–37)	44.9* (36.9–53.9)	26 ± 3	47 ± 4	25 ± 9	46 ± 7
Cu	11 (9–13)	30* (24–36)	9.5 ± 2	30 ± 4	10 ± 3	30 ± 6
Zn	104 (101–113)	223 (213–233)	94 ± 13	189 ± 15	90 ± 22	193 ± 50
As	13.4 (12.5–14.2)	27.6 (24.7–30.5)	13.4 ± 1.4	28.2 ± 1.5	13 ± 3	28 ± 5
Sb	1.7 (1.4–1.8)	1.31* (1.19–1.43)	1.7 ± 0.2	1.29 ± 0.09	1.6 ± 0.4	1.2 ± 0.2
Cd	1.3* (1.1–2.7)	0.26* (0.21–0.31)	1.20 ± 0.15	0.26 ± 0.03	1.2 ± 0.3	0.21 ± 0.09
Pb	60 (55–71)	37.7* (30.3–45.1)	49 ± 7	32 ± 9	60 ± 14	37 ± 8

Note: Results are reported with their confidence level of 95%. The table also reports the certified values and the 95% confidence intervals for IAEA Soil-7 and SL-1 (n = number of independent replicates).

*Information value.

solution; hence the ICP-MS procedure used in this study needs further investigation.

The Pb mass fraction value measured by method A in IAEA Soil-7 is negatively biased. Low recoveries for Pb (81 and 85%) using method A may be related to the internal standard used. Rh is not suitable to minimize the instrumental signal fluctuation and matrix effects. The comparison between the results obtained with methods A and B shows that there is no a significant difference between the results obtained by these methods, with the exception of Pb in IAEA Soil-7. The results reported in Table 3 show that the standard deviations are generally lower for method A than in method B. This could be explained by the difference in total energy input used in the digestion steps. Method A uses a higher total energy input (5,265 kJ) than method B (3,192 kJ).

Table 4 reports the results obtained with method C in LGC-6187, certified for aqua regia method, and in BCR-141R certified for aqua regia extractable elements and for the total element mass fractions. The uncertainties reported for the CRMs represent the 95% confidence limit. The standard deviations associated with the measured values are reported with an approximate level of confidence of 95%. Generally, the repeatability for method C is lower for BCR-141R than for LGC-6187. This could be due to higher homogeneity of BCR-141R in comparison with that of the LGC-6187 certified reference material. The element mass fractions obtained on LGC-6187 were significantly higher for As, Cr and Ni, while on BCR-141R only Cr and Co are positively biased in comparison with element mass fractions certified using aqua regia. For Zn, Cu, Cd, and Pb the results with their standard deviation do not clearly overlap the 95% confidence

Table 4 Element mass fractions for digestion method C applied to LGC-6187 and BCR 141R CRMs

	LGC6187 Certificate aqua regia soluble mass fraction (mg kg ⁻¹)	BCR 141R Certificate aqua regia soluble mass fraction (mg kg ⁻¹)	Certificate total mass fraction (mg kg ⁻¹)	Measured mass fractions			
				Method C HCl + HNO ₃ + H ₂ O ₂			
				LGC6187 (mg kg ⁻¹)	(n = 10), CV %	BCR 141R (mg kg ⁻¹)	(n = 4), CV %
Cr	84 ± 9.4	138 ± 5	195 ± 7	101 ± 14	6.8	155 ± 7	2.4
Co		9.2 ± 0.5	10.5 ± 0.4			10.1 ± 0.1	0.5
Ni	34.7 ± 1.7	94 ± 5	103 ± 3	41 ± 8	9.3	93 ± 8	4.3
Cu	83.6 ± 4.1	46.9 ± 1.8	46.4 ± 1.8	94 ± 13	6.8	46.4 ± 0.2	0.2
Zn	439 ± 26	270 ± 8	283 ± 5	455 ± 19	2.1	261 ± 6	1.1
As	24 ± 3.2			29 ± 2	3.1		
Cd	2.7 ± 0.3	14 ± 0.4	14.6 ± 0.5	2.9 ± 0.4	6.1	13.6 ± 0.2	0.6
Pb	77.2 ± 4.5	51.3 ± 2	57.2 ± 1.2	82 ± 4	2.2	50.7 ± 0.6	0.6

Note: Results are reported with their confidence level of 95%. The table also reports the certified values for extractable metals, total mass fraction and their uncertainties. The uncertainty represents the half-width of the 95% confidence interval (n = number of independent replicates).

interval of the recommended values. To check if the mass fraction values of these elements in LGC-6187 are biased, the criterion reported in ISO Guide 33 [27] has been used. On the basis of this criterion, that compares the bias with the uncertainty of the certified value combined with the standard deviation of the measurement process, Zn, Cu, Cd and Pb mass fractions are in good agreement with the certified values. The same ISO criterion was applied on the results obtained in BCR-141R. Zn mass fractions is in agreement in comparison with the certified value for aqua regia soluble mass fraction.

The comparison between the certified total element contents in BCR-141R and the values obtained with method C show that values for Cr and Co are negatively biased. The application of ISO criterion shows that measured Zn value is negatively biased.

The results reported above could be explained by the difference in measurement procedures used in this study, compared to those used for CRM certification. The degree of dissolution of solid sample with aqua regia depends on the input of energy. Method C uses a different power input and a different reaction time during the digestion step from those used in the certification of LGC-6187 (conventional heating under open reflux condition) and of BCR-141R. H₂O₂ used in method C enhances the dissolution of the organic mass fraction. To investigate the effects induced by the procedure used in this work, LGC-6187 was digested using microwave assisted aqua regia, without the addition of H₂O₂ (Table 5). In this case the mass fractions of Cr, Ni, Cu and As are in agreement with the certificate values, while the measured Zn mass fraction is negatively biased. This confirms the need of further investigation on the measurement of Zn by ICP-MS.

Comparison of the three digestion methods for measuring elements in agricultural soil samples

The homogeneity of the soil test samples was verified measuring by INAA 10 test portions from three different test

Table 5 Element mass fractions for assisted aqua regia digestion method applied to measurement of LGC-6187 CRM

	Method aqua regia (ISO11466*)	
	LGC6187 (mg kg ⁻¹)	(n = 3) Recovery %
Cr	92 ± 3	110
Ni	36 ± 2	104
Cu	86 ± 2	103
Zn	390 ± 56	89
As	25.1 ± 0.5	105

Note: Results are reported with their experimental standard deviations with a coverage factor of 2 to yield an approximate confidence level of 95% (n = number of independent replicates).

*Microwave-assisted digestion method.

Table 6 ICP-MS results on eight agricultural soil samples collected in a reference site in Italy

Soil sample	Cr		Co		Ni		Cu		Zn		As		Cd		Sb		Pb	
	A+B* (n=6) (mg kg ⁻¹)	C** (n=3) (mg kg ⁻¹)	A+B (n=6) (mg kg ⁻¹)	C** (n=3) (mg kg ⁻¹)	A+B (n=6) (mg kg ⁻¹)	C (n=3) (mg kg ⁻¹)	A+B (n=6) (mg kg ⁻¹)	C (n=3) (mg kg ⁻¹)	A+B (n=6) (mg kg ⁻¹)	C (n=3) (mg kg ⁻¹)	A+B (n=6) (mg kg ⁻¹)	C (n=3) (mg kg ⁻¹)	A+B (n=6) (mg kg ⁻¹)	C (n=3) (mg kg ⁻¹)	A+B (n=6) (mg kg ⁻¹)	C (n=3) (mg kg ⁻¹)	A+B (n=6) (mg kg ⁻¹)	C (n=3) (mg kg ⁻¹)
1	119.9	60 ± 2	13 ± 1	13.3 ± 0.2	47 ± 4	50 ± 6	42 ± 5	43 ± 1	95 ± 6	10.8 ± 0.7	12.0 ± 0.1	0.66 ± 0.05	0.66 ± 0.03	1.26 ± 0.13	0.660 ± 0.003	36 ± 3	38 ± 2	
2	122 ± 27	57 ± 2	13 ± 2	12.3 ± 0.5	49 ± 4	46 ± 3	35 ± 4	34 ± 1	94 ± 11	11.2 ± 0.3	11.2 ± 0.3	0.68 ± 0.12	0.66 ± 0.01	1.36 ± 0.24	0.7 ± 0.1	38 ± 7	31.4 ± 0.7	
3	123 ± 24	59 ± 1	13 ± 1	12.9 ± 0.2	48 ± 7	47 ± 2	46 ± 5	47.2 ± 0.5	91 ± 10	11.1 ± 0.9	11.9 ± 0.3	0.62 ± 0.08	0.64 ± 0.01	1.24 ± 0.10	0.66 ± 0.05	34 ± 2	31 ± 1	
4	116 ± 21	62 ± 7	12 ± 1	12.3 ± 0.3	45 ± 4	48 ± 3	44 ± 4	46 ± 1	87 ± 12	11.2 ± 1.5	12.1 ± 0.2	0.60 ± 0.15	0.6 ± 0.1	1.27 ± 0.23	0.67 ± 0.02	32 ± 3	29.7 ± 0.6	
5	114 ± 22	62 ± 4	13 ± 1	12.9 ± 0.2	49 ± 9	48 ± 2	38 ± 4	39.2 ± 0.9	88 ± 10	10.6 ± 1.2	11.3 ± 0.4	0.58 ± 0.12	0.58 ± 0.03	1.23 ± 0.23	0.61 ± 0.03	30 ± 3	29 ± 1	
6	109 ± 39	50 ± 3	11 ± 1	10.6 ± 0.6	42 ± 6	39 ± 2	31 ± 4	31 ± 1	79 ± 9	9.6 ± 0.3	9.9 ± 0.6	0.57 ± 0.08	0.55 ± 0.02	1.13 ± 0.13	0.57 ± 0.04	28 ± 1	24 ± 1	
7	104 ± 20	49 ± 4	11 ± 1	10.5 ± 0.7	42 ± 12	39 ± 2	37 ± 8	36 ± 2	78 ± 11	9.5 ± 0.4	9.9 ± 0.5	0.54 ± 0.06	0.55 ± 0.02	1.17 ± 0.11	0.55 ± 0.05	29 ± 2	27 ± 1	
8	84 ± 15	42 ± 3	9 ± 1	9.0 ± 0.5	35 ± 12	31.0 ± 0.2	29 ± 6	28.8 ± 0.2	69 ± 6	8.3 ± 0.9	8.7 ± 0.6	0.54 ± 0.10	0.51 ± 0.02	0.99 ± 0.09	0.49 ± 0.05	26 ± 1	23 ± 1	

Results obtained after digestion with methods A and B are pooled and reported as A + B. Column C reports results obtained after digestion with method C. Results are reported with their experimental standard uncertainty, with a coverage factor of 2 to yield an approximate confidence level of 95% (n = number of independent replicates).

A + B* = results from method A (HNO₃ + HF) pooled with results from method B (HCl + HNO₃ + HF).

C** = results from method C (HCl + HNO₃ + H₂O₂).

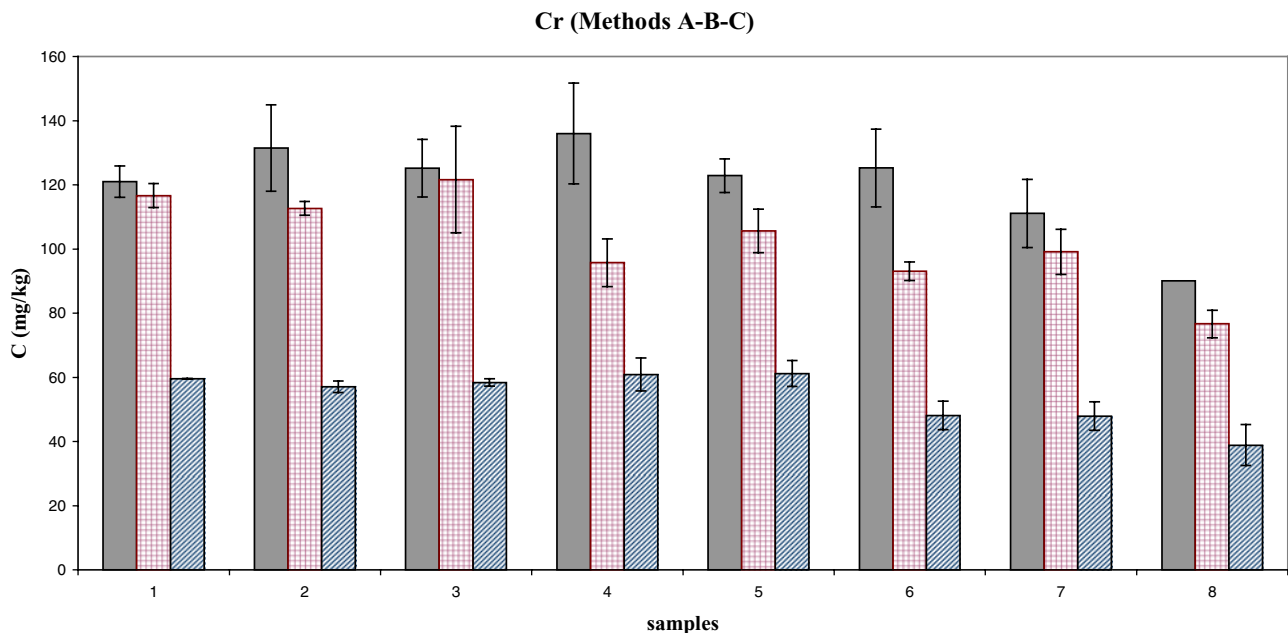


Fig. 1 Cr results on eight agricultural soil samples collected in a reference site in Italy determined by ICP-MS after acid digestion by methods A, B, and C (grey method A; squared method B; striped method C). The uncertainty represents the standard deviation of three replicates

samples. The relative standard deviation for all elements (As, Co, Cr, Sb and Zn) was less than 4%. These results indicate that the differences of measurement results using different methods can be detected when the differences are higher than the residual heterogeneity in the test samples.

In the soil samples, the better precision of method A in comparison with method B is not confirmed. As an example, precision for As ranges from 1 to 14% (method A) and from 1 to 6% (method B), while for Cr ranges from 1 to 13% (method A) and from 2 to 16% (method B). This seems to point to a different sample behavior of the agricultural soils and the RMs (IAEA Soil 7 and IAEA SL1), possibly due to the different matrices and different levels of homogeneity in RMs and in test samples used in this investigation.

The results for As, Cd, Co, Cr, Cu, Ni, Pb, Sb and Zn using methods A and B on the eight agricultural soils are pooled on the basis of studies of RMs, revealing a similar behavior. The results obtained by means of methods A and B are compared with those measured after sample extraction by method C (Table 6). All the results are reported with their experimental standard uncertainty, with a coverage factor of 2 to yield an approximate confidence level of 95%.

Analysis of variance using the ANOVA test at a confidence level of $\alpha = 0.05$ was performed to assess the significance of differences among the three methods. ANOVA shows no significant differences for As, Cd, Co, Cu, Ni, Pb and Zn. These results suggest that for the soil analyzed the partial

digestion method aqua regia leads to results equivalent to those of the total digestion methods.

For Cr, the ANOVA test shows a statistically significant difference among the methods A-B-C, attributable to method C. As can be easily recognized from Fig. 1 and Table 6, Cr mass fractions determined by using method C are lower by a factor of two than by using methods A and B. This could be due to the presence of insoluble refractory Cr minerals which cannot be dissolved without using HF. Similar low recoveries (23–74%) for Cr using an aqua regia digestion procedure are described in the literature for river sediments and soils [28, 29].

The ICP-MS results on samples digested by using method C for Sb (Fig. 2 and Table 6) show mass fraction values about 50% lower than those obtained by using method A and B. These results could be due to a not efficient extraction of Sb normally bound to silicates.

A *t*-test at a confidence level of $\alpha = 0.05$ between results obtained by methods A and B showed no significant difference for Cr and Sb.

Comparison of ICP-MS measurements results with INAA in measuring elements in agricultural soils

Among the trace elements selected in this investigation, As, Zn, Co, Cr and Sb were measured by INAA as well. Table 7 reports the grand mean of As, Zn, Co mass fraction values obtained by ICP-MS after the soil digestion with methods A, B and C, as well as the INAA analytical results for the eight agricultural soils investigated. INAA data are re-

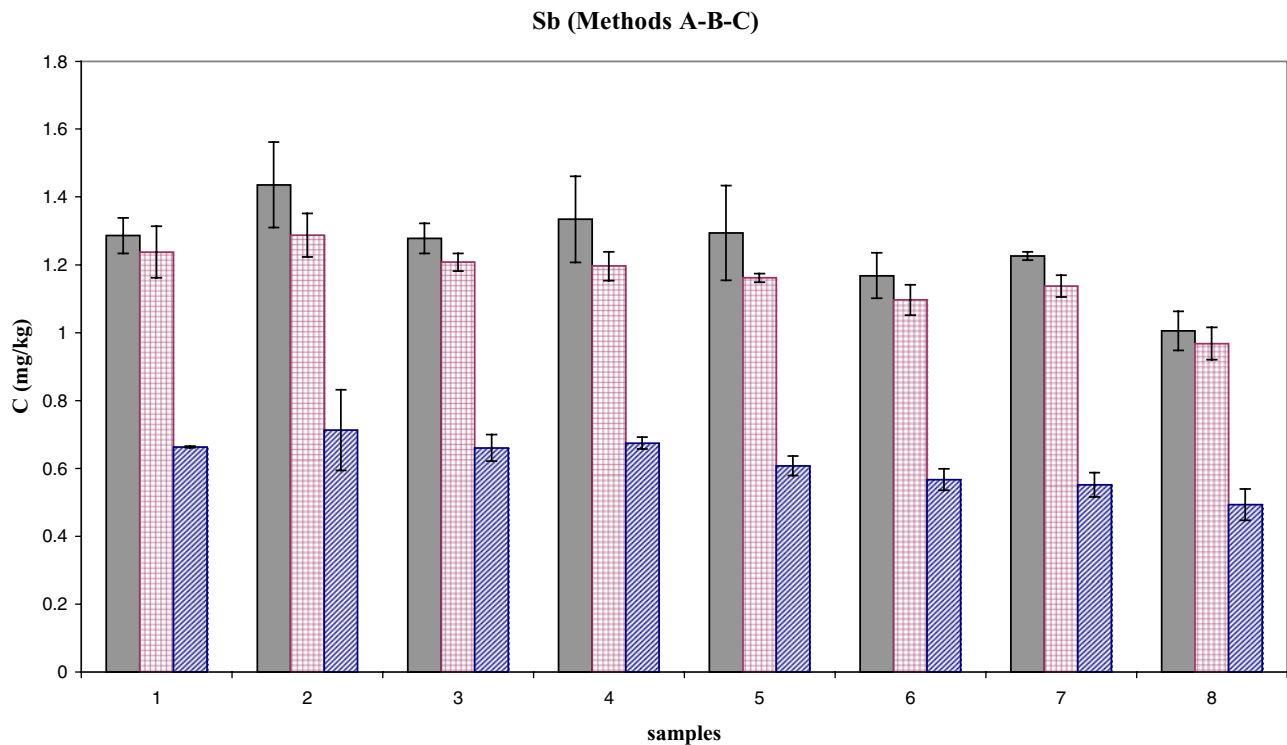


Fig. 2 Sb results on eight agricultural soil samples collected in a reference site in Italy determined by ICP-MS after acid digestion by methods A, B, and C (grey method A; squared method B; striped method C). The uncertainty represents the standard deviation of three replicates

ported with their standard deviation from counting statistics in gamma spectrometry. The comparison of k_0 -INAA results with those obtained by ICP-MS are in good agreement for all elements except for Zn that is slightly underestimated. This result confirms the need for future study of Zn measurement by ICP-MS. Looking at the results obtained after the digestion with method A, Cr mass fractions in soil are lower than k_0 -INAA values (Table 8). These results are attributable to an incomplete digestion of Cr, bound to residual fraction as

refractory mineral, even when using HF. Yang et al. [24] reported low recoveries for Cr in a certified reference material, HISS (sediment), using ICP-MS and closed vessel digestion with HF and HNO_3 . The authors obtained mass fraction values which were almost a factor three lower than the value found by INAA. Sb mass fraction values in ICP-MS measurements are slightly higher than INAA measurements. The presence of HF in the digestion mixture, in this case, is sufficient to completely digest silicate bound Sb.

Table 7 ICP-MS and INAA results on agricultural soil samples collected at a reference site in Italy

Soil sample	As (mg kg^{-1})		Zn (mg kg^{-1})		Co (mg kg^{-1})	
	ICP-MS Grand mean methods A, B, C* ($n = 3$)	INAA ($n = 1$)	ICP-MS Grand mean methods A, B, C ($n = 3$)	INAA ($n = 1$)	ICP-MS Grand Mean methods A, B, C ($n = 3$)	INAA ($n = 1$)
1	11 ± 1	11 ± 1	95 ± 4	101 ± 8	13.0 ± 0.9	13 ± 1
2	11 ± 1	11 ± 1	91 ± 12	97 ± 8	12.5 ± 0.4	13 ± 1
3	11.4 ± 1.1	11 ± 1	90 ± 5	99 ± 8	12.9 ± 0.2	13 ± 1
4	11.5 ± 1.4	12 ± 1	88 ± 3	90 ± 7	12.1 ± 0.3	12 ± 1
5	10.8 ± 0.9	10.7 ± 0.8	89 ± 3	89 ± 7	12.7 ± 0.5	12 ± 1
6	9.7 ± 0.5	9.0 ± 0.7	80 ± 5	83 ± 7	10.9 ± 0.5	11 ± 1
7	9.6 ± 0.5	9.5 ± 0.8	77 ± 9	85 ± 7	10.7 ± 0.5	11 ± 1
8	8.4 ± 0.5	8.7 ± 0.7	68 ± 2	73 ± 6	9.1 ± 0.4	9.5 ± 0.8

Note: Grand means of data obtained after digestion with method A, B, and C. Results are reported with their standard deviations at a confidence level of approximately 95% ($n =$ number of independent replicates).

Methods A, B, C * = grand mean of the ICP-MS results after digestion with method A ($\text{HNO}_3 + \text{HF}$), B ($\text{HCl} + \text{HNO}_3 + \text{HF}$) and C ($\text{HCl} + \text{HNO}_3 + \text{H}_2\text{O}_2$).

Table 8 Cr and Sb mass fractions on agricultural soil samples collected in a reference site in Italy

Soil sample	Cr (mg kg ⁻¹)		Sb (mg kg ⁻¹)	
	ICP-MS Method A* (n = 3)	INAA (n = 1)	ICP-MS Method A (n = 3)	INAA (n = 1)
1	121 ± 10	223 ± 19	1.3 ± 0.1	1.2 ± 0.1
2	132 ± 27	260 ± 21	1.4 ± 0.3	1.2 ± 0.1
3	125 ± 18	215 ± 19	1.28 ± 0.09	1.3 ± 0.1
4	136 ± 31	215 ± 19	1.3 ± 0.3	1.3 ± 0.1
5	123 ± 10	219 ± 19	1.3 ± 0.3	1.2 ± 0.1
6	125 ± 24	234 ± 21	1.2 ± 0.1	1.1 ± 0.1
7	111 ± 21	250 ± 21	1.23 ± 0.02	1.1 ± 0.1
8	90 ± 2	224 ± 19	1.01 ± 0.08	1.0 ± 0.1

Note: Comparison between the results obtained with method A (a) and ICP-MS and INAA results (b). Results are reported with their standard deviations at a confidence level of about 95% (*n* = number of independent replicates).

Method A* = HNO₃ + HF.

Conclusions

For methods A and B, results for Co, Ni, Cu, As, Sb and Cd were found in a very good agreement, while for method C the results for, Cu, Cd and Pb corresponded well with the certified values of the selected RMs.

Both methods A and B generally showed a good repeatability standard deviation. The higher precision of method A is found only in the case of the RMs and not for the real soil samples analyzed. Concerning method C, the results on LGC-6187 show higher repeatability than those on BCR141R.

Modified digestion conditions, occurring in method C, in terms of energy input of microwave, resulted in positively biased values for Cr element for both CRMs certified for aqua regia soluble mass fraction (LGC-6187 and BCR141R). Cd, Pb and Cu mass fraction values are in agreement with the certified values in both CRMs. Zn results are generally negatively biased for all methods. Zn is generally brought into solution, thus the ICP-MS procedure used in this study needs further investigation.

ANOVA was performed on the ICP-MS results obtained after the application of the three digestion methods on eight agricultural soil samples, collected in one test area. The only significant differences are related to Cr and Sb results obtained after extraction with method C (modified aqua regia). This could be due to the presence in the soil of insoluble Cr minerals and Sb bound to silicates.

k₀-INAA analytical results of the soil samples were compared with ICP-MS measurements on the same test samples. The results of As and Co are in good agreement regardless which of the three digestion methods is used. In this case, the procedures used do not affect the final analytical results.

Cr results, even if obtained with “total” digestion method with the best recovery (method A), are lower by a factor of 2 than the INAA results. Sb after digestion with HNO₃ + HF is in good agreement with INAA results.

In conclusion, the results reported in this paper highlight that the digestion procedure is an essential part of the definition of the measurement procedure in environmental analysis.

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